Influence of Microbial Contamination in Clean Rooms Affecting the Validation of Cleaning Procedures.

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Spacecraft components used in NASA life detection missions need to be cleaned of any terrestrial biological/organic contamination. Cleaning technologies are under development to reach this objective. The purpose of our studies is to determine how and where contaminants are introduced to these cleaning technologies, thereby affecting validation of cleaning procedures. There were 3 steps involved in the cleaning process examined: pre-cleaning, cleaning and validation. In addition to human participation and the cleaning environment as the sources of contamination, materials such as solvents, wipes, and instruments used were also considered. The evaluation of the cleaning technologies was carried out by artificially inoculating a known microbial species (*Bacillus subtilis* spores) onto various metal surfaces. The 5 different cleaning methods tested during this study involved manual, semi-automated, and automated steps. Spacecraft qualified materials cleaned were Aluminum (6061, 7075), Chromated aluminum (7075), and Titanium (6Al-4V). Appropriate controls were included to trace sources of contamination. Contaminants were defined as any microbial growth other than the characteristic colony morphology of *B. subtilis* spores. Representatives of various microbial contaminants were selected and purified based on color, morphology, and growth rate. All isolates were initially stained for the Gram-reaction and spore staining to determine the kinds of microbes. The BioLog identification system was used to classify the isolates into groups. The members of each group were selected for species identification by 16S rDNA analysis. The results showed that the microbial contamination was introduced in the pre-cleaning steps of the cleaning processes. In contrast to the fully automated process, both manual and semi-automated cleaning processes added microbial contamination. Irrespective of the materials cleaned, contamination was observed. The majority of microbial contaminants were Gram-positive bacteria as well as Yeast-Fungi groups.